Neuroendocrinology Letters Volume 37 No. 3 2016
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

Evaluation of the analgesic effect of morphine on models of acute nociceptive pain in rats with a central noradrenergic system lesion

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Submitted: 2016-01-23 Accepted: 2016-03-03 Published online: 2016-07-27

Key words: **morphine; acute pain; rats; central noradrenergic; nociception**

Neuroendocrinol Lett 2016; **37**(3):239–244 **PMID:** 27618597 NEL370316A08

Abstract

OBJECTIVES: Stimulation of some noradrenergic system receptors demonstrates a synergistic anti-nociceptive effect with the opioid system at the level of peripheral tissues, spinal cord, and supraspinal structures. Furthermore, opioids stimulate the noradrenergic descending pathways originating from the substantia nigra by presynaptic inhibition of the GABA neuron ends.
It is thus important to determine whether a disruption to the adrenergic transmission obtained via DPS-4 administration to neonatal rats impacts the perception of noxious stimuli mediated by 5-HT3-serotonin receptors at the level of spinal cords or higher tiers of the central nervous system.
DESIGN & SETTING: The studies were conducted with neonatal and adult rats, males of the Wistar strain in which a central noradrenergic system lesion was induced with DSP-4 on days 1 and 3 of life. Next, the evaluation of the analgesic effect of morphine was performed on 8- to 10-week-old animals using the following models of acute nociceptive pain: the hot plate test and the tail immersion test as models of acute nociceptive pain induced by a thermal stimulus, and the paw withdrawal test as a model of nociceptive pain caused by a mechanical stimulus.
RESULTS: Morphine was found to produce a longer-lasting analgesic effect in the tail immersion test in the control group than in rats. Similarly, in the paw withdrawal test, this substance generated a strong analgesic effect (with over 200% of analgesia) in the control group, whereas its action in the rats with DSP-4 lesions was statistically significant. Morphine induced analgesia at about 13–14% in the control rats when examined with the hot plate test.
CONCLUSIONS: The disruption to the central noradrenergic system in an early stage of development resulted in a reduction of the analgesic effect of morphine in the models of acute pain in which the mechanisms of supraspinal perception are involved.

ORIGINAL ARTICLE

INTRODUCTION

Nociceptive pain is caused by stimulation of the pain receptors (nociceptors) in the peripheral nervous system, which triggers signals transmitted by two types of neural fibers: fast, myelinated Ad axons, and slow, non-myelinated C fibers. They go towards the spinal cord, creating the dorsolateral tract (*tractus dorsolateralis),* within which there are shorter descending branches and slightly longer, ascending ones. However, their length does not exceed two or three spinals segments and their course finishes at the marginal zone cells and gelatinous substance and in the head of caudal horn. The A d and C type fibers conduct a noxious stimulus to the neuronal body located in the dorsal spinal root ganglion (Wordliczek *et al.* 2004).

The C-type fibers are very thin and sensitive to damage, although they do not have myelin sheaths and conduct pain stimuli very slowly. Their arousal triggers a progressively increasing blunt or burning pain. These fibers are very numerous and branching, innervating in general large body areas, which makes a diseased individual able to provide only a rough localization of pain conducted by them (Schmidt *et al.* 1997). They conduct electrical impulses induced by mechanical, thermal and chemical stimuli. At their ends, there are different types of receptors, of which the opioid ones are predominant (Stein 1995). These receptors are inactive until aroused by a number of substances released from damaged tissues such as bradykinin, histamine, 5-HT, ATP, potassium ions, leukotrienes, and prostaglandins (Przewłocka 2004), which cause the release of opioids acting on the opioid receptors.

The Ad-type fibers are thin, myelinated and responsible for conducting the impulses that are perceived as stabbing pain. They conduct stimuli from smaller body areas than the C fibers, which makes the pain easier to locate by a patient. There are virtually no opioid receptors on the Ad-type fibers and the nociceptors found on these ends are always at "a ready and alerted state". Thus, the activities of these receptors are modified pharmacologically only to a very limited degree (Schmelz *et al.* 1997; Boucher *et al.* 2001).

The lateral spinothalamic tract (*tractus spinothalamicus lateralis)* is the next stage of pain conduction. The fibers therein originate mainly in the cells of the caudal spinal horns and they then cross with the fibers of the contralateral side in the central intermediate substance. The spinothalamic fibers of each segment, having entered the contralateral half of the spine, are laid as a thin lamella within the lateral cord outside of the grey matter, thereby moving the fibers from the lower segments to the periphery and caudally. Such an arrangement is repeated in all spinal segments, creating a concentric system of spinothalamic fibers. The fibers conducting stimuli from the perineum and hind limbs are situated the most superficially, while the ones conducting stimuli from the trunk, front limb and neck are laid deeper, which means that there is a somatotropic localization in the spinothalamic pathways. The lateral spinothalamic tract runs from the spinal cord to the medulla oblongata and further, via the pons and the midbrain, to the thalamus. Over the distance up to the medulla oblongata (in the brainstem), it is called the spinal lemniscus (Dobrogowski *et al.* 2004).

The spinothalamic tract constitutes the so-called second neuron of the great system of pain, heat and cold perception, which starts in the receptors and finishes in the brain cortex. The first neuron (peripheral) is composed of the spinal ganglia cells with inward projections entering into the spinal cord via the lateral bundle of the dorsal root, whereas the third neuron consists of the thalamic cells (ventral caudolateral nucleus) sending its axons to the brain cortex (Keele 1957).

The additional pain-conducting tracts associated with its perception also include the spinocervical tracts *(tractus spinocervicalis)*. This tract ends in the lateral cervical nucleus (*nucleus cervicalis lateralis)*, which is much smaller in humans than in other mammals (Bushnell *et al.* 2006).

When the receptors located at the ends of sensory nerves are aroused, a pain signal (an electric impulse) travels towards the caudal spinal horns and reaches the synaptic ends where the neurotransmitters accumulated in the vesicles, such as glutamate and substance P, are released (Stucky *et al.* 2001; Hao *et al.* 1997; Ren *et al.* 1999). These mediators are secreted into the synaptic gap, where they bind to the appropriate receptors and stimulate them. The most important post-synaptic receptors mediating pain perception include: NMDA *(N-methyl-D-aspartate)* receptors, AMPA *(α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid)* receptors, and NK-1 for substance P. the pre-synaptic receptors, such as the opioid m and d receptors, α_2-adrenergic receptors, $GABA_B$ and $5\text{-}HT_2$ and $5\text{-}HT_3$, exert a modulating effect on the release of glutamate and substance P. The opioid receptors are situated in both the pre-synaptic and post-synaptic membrane, although in a smaller amount in the latter. The above-mentioned systems of neurotransmitters may be thus a target site for pharmacological modification of pain conduction (Ren *et al.* 2002).

When exposed to a strong or repeated peripheral nociceptive stimulus, the complex processes of pain conduction in the spinal cord are activated. On one side, the endogenous substances inhibiting pain perception are released, while the mediators increasing and maintaining the pain are secreted. The activation of NMDA receptors is crucial for the process of "spinal sensitization", as these receptors are responsible both for perpetuating the pain and reducing the sensitivity to opioids (Kreitler *et al.* 2007).

The midbrain and medulla oblongata are another location suitable for the potential modulation of pain conduction. They give rise to the descending pathways which either perpetuate or halt pain conduction in the

medulla oblongata. The serotoninergic and noradrenergic pathways play an important role in the mechanism of pain inhibition at this level (Snider *et al.* 1998; Roczniak *et al.* 2010).

Based on the available studies, it is believed that the serotoninergic and adrenergic systems cooperate in modulating pain at the spinal cord level (Pertovaara 2006; Roczniak *et al.* 2013; Paul *et al.* 2001). The conducted studies thus included the model of the central noradrenergic system damage (lesion) with DSP-4 developed in the Department of Pharmacology in Zabrze (Bortel *et al.* 2008).

The objective of the study was to determine whether chemical damage to the central noradrenergic system in an early phase of the individual development impacts the analgesic effect of morphine in the models of acute nociceptive pain in rats.

METHODOLOGY AND MATERIALS

Animals

The studies were carried out with neonatal and adult rats, males of the Wistar strain, aged 8–10 weeks. The animals were kept in a room with constant temperature (app. 22 °C) and the light day/night cycle: 12 h/12 h (light provided from 7 am to 7 pm). Throughout the study, the animals were given free access to water and a standard ration.

The study was approved by the Local Ethical Committee at the Medical University of Silesia (The Approval No. 66 of 11.12.2007).

The method used to produce a lesion in the central noradrenergic system (Jaim-Etcheverry 1998; Dooley et al. 1983)

The male neonatal rats were divided into 2 groups:

Group I: control. The animals were administered zimelidine dihydrochloride at 10 mg/kg BW SC in a volume of 1.0 mL/kg BW and after 30 minutes a 0.9% NaCl solution at 1.0 mL/kg BW SC on days 1 and 3 of life.

Group II: DSP-4. The animals were administered zimelidine dihydrochloride at 10 mg/kg BW SC in a volume of 1.0 mL/kg BW and after 30 minutes DSP-4 at 50 mg/kg BW SC on days 1 and 3 of life.

The behavioral studies were performed on adult, 8- to 10-week-old animals. Morphine dosing was set based on personal studies and literature data.

All behavioral assessments were carried out from 8am to 3pm with the individual investigated groups consisting of 8–10 animals.

Evaluations of an analgesic effect of morphine on the models of acute nociceptive pain

The hot plate test: a model of nociceptive pain induced by a thermal stimulus (O'Callaghan *et al.* 1975)

Following a 30-minute adaptation period to the conditions in a room in which the experiment was performed, the animals were placed inside a test cage with a copper plate floor, 400×300×42 mm in size, heated to 56 °C ("hot plate" Hp41, COTM, Białystok). At the moment of putting a rat inside the cage, a stop-watch was switched on. The timer was switched off when one of the following behaviors was noted: shaking or licking of the paws, taking the paws off the floor, jumping or squealing. This served to determine the latency time (in seconds) before the investigated substance was administered (T_o), namely, the time that elapsed from the (pain) stimulus till the reaction to it. The measurement was taken 10 minutes after the injection of 1.0 mL 0.9% NaCl in the animals from both groups. Morphine was then administered (7.5 mg/kg BW SC) and the latency time was again recorded after 20, 40, 60, and 80 minutes following the injection. 20 seconds after placing a rat in the cage, if any reaction was seen to the applied thermal stimulus, the test was stopped to prevent any damage to the tissues (burning of the foot pads). Based on the findings, the percentage (%) of analgesia was then calculated with the following formula:

$$\% \text{ analgesia} = [(T_x - T_o) / (T_{max} - T_o)] \times 100$$

where:

T_x – individual latency time in a specific time range following the administration of a given substance

T_o – individual latency time before a given substance is administered

T_{max} – 20 s

The tail immersion test: a model of nociceptive pain induced by a thermal stimulus (Janssen *et al.* 1963)

In order to minimize the impact of stress caused by immobilization on the results generated in the tail immersion test, the rats were subjected to training the day before the actual test. This training was aimed at adapting the animals to a test cage in which they had a limited range of unrestrained movement, such as changing the body position along the vertical axis, during the study.

Throughout the adaptation procedure, the rats were placed individually in cages made of Plexiglas, 220×70×70 mm in size, for about 30 minutes with the tail left outside the cage.

Next day, the test was performed. To this end, the rats were put inside the same cages and, after 15 minutes, the latency time was recorded from the moment the tail was immersed in a cylinder filled with water at 58 °C till the reaction was noted involving a rapid movement of the tail in response to the applied thermal stimulus (the so-called tail-flick). It should be mentioned that unless the above-mentioned reaction was seen within 10 seconds from the moment of tail immersion in the cylinder with water, the test was stopped. Such an approach prevented any thermal damage to the tissues.

Each rat was then administered morphine (7.5 mg/kg BW SC) and the latency time was again measured after 20, 40, 60, and 80 following the injection. Based on the findings, the percentage (%) of analgesia was then calculated with the following formula:

$$\% \text{ analgesia} = [(T_x - T_o) / (T_{max} - T_o)] \times 100$$

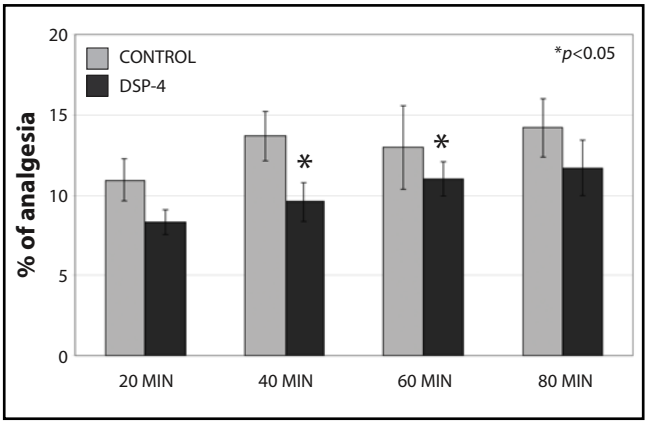

Fig. 1. Impact of the DSP-4 lesion on the analgesic effect of morphine (7.5 mg/kg BW SC) in the hot plate test in rats ($\bar{x}$=SEM; n=10)

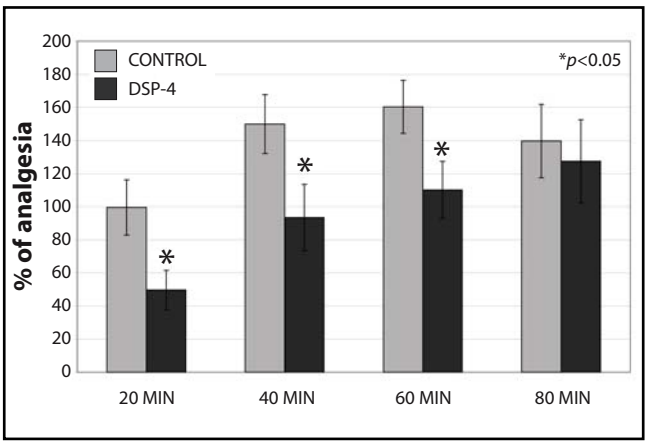

Fig. 2. Impact of the DSP-4 lesion on the analgesic effect of morphine (7.5 mg/kg BW SC) in the tail immersion test in rats ($\bar{x}$=SEM; n=10)

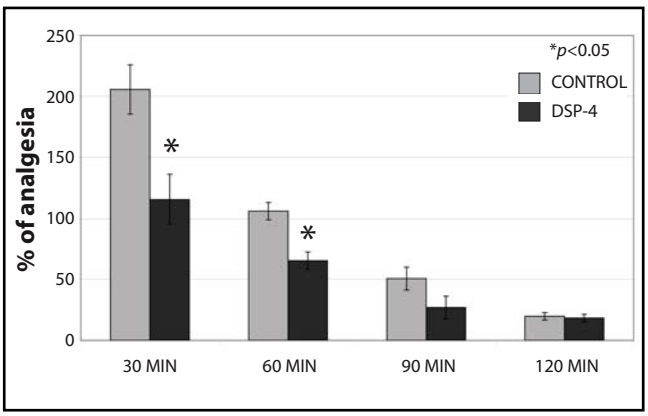

Fig. 3. Impact of the DSP-4 lesion on an analgesic effect of morphine (7.5 mg/kg BW SC) in the paw withdrawal test in rats ($\bar{x}$=SEM; n=10).

where:

T_x – individual latency time in a specific time range following the administration of a given substance

T_o – individual latency time before a given substance is administered

T_{max} – 10 s

The paw withdrawal test (Randall-Selitto test): a model of nociceptive pain induced by a mechanical stimulus (Simth et al. 2007)

The test was performed using an Ugo Basile-type analgesia meter (Italy). After 30 minutes of the administration of 0.9% NaCl at 1.0 mL/kg IP, the right hind limb was placed (with the foot pad down) on a circular surface made of Plexiglas, Ø 1 cm. Next, the dorsal aspect of the paw was squeezed with a blunt-ended cone attached to a mechanical lever that allowed placing an increasing linear mechanical pressure. The observations were performed by reading the pressure force (expressed in grams) at which a rat squealed or tried to remove the paw rapidly from beneath the bolt. The procedure was repeated 3 times at 10-minute intervals and the mean was calculated from the measurements. Next, the rat was administered morphine at 7.5 mg/kg BW SC, both in the control group and in the treatment group (with the noradrenergic system lesion). After 30 minutes following the injection of investigated substances, the measurements were retaken according to the above described schedule. The test was carried out every 30 minutes for 120 minutes. The percentage of analgesia was calculated with the following formula:

$$\% \text{ analgesia} = [(100 \times B)/A] - 100$$

where:

A – pressure force expressed in grams, following the administration of a 0.9% NaCl solution (the mean of 3 measurements)

B – pressure force expressed in grams at respective 30-minute intervals following the injection of morphine (7.5 mg/kg BW SC)

RESULTS

Morphine at 7.5 mg/kg BW SC, administered during the hot plate test, induced analgesia at approximately 13–14 % (Figure 1).

For the tail immersion test, morphine at 7.5 mg/kg BW SC produced a stronger analgesic effect in the control group than in the DSP-4 rats. The differences were statistically significant at 20, 40, and 60 minute of the test (Figure 2).

Morphine, used as an analgesic substance at the dose of 7.5 mg/kg BW S.C. in the paw withdrawal test, generated a strong analgesic effect (with over 200% of analgesia) in the control group whereas its action was statistically significantly weaker in the DSP-4 lesion rats (Figure 3).

DISCUSSION

Based on their potency and mechanism of action, analgesic medicines are divided into two basic groups: narcotics (opiates) of plant origin, semisynthetic or synthetic and analgesic drug with antipyretic properties (and anti-inflammatory effects), namely non-steroidal anti-inflammatory drugs (NSAID) (Gomółka 1998).

As an phenanthrene alkaloid, morphine is the main substance of opiates found in opium. Opioids operate via specific receptors located in the brain. Today, there are three types of classical opioid receptors known: MOR (mu, m), DOR (delta, d) and KOR (kappa, k) receptors and one non-classical NOR (ORL1, *opioid receptor like*) receptor, with a number of subtypes distinguished within the types (Kieffler *et al.* 2002). Several opioid peptides are the endogenous ligands of these receptors, of which the most investigated ones include: enkephalins, endorphins and dynorphins. Narcotic analgesic medicines demonstrate very high activity and pharmacological specificity and are thus used to treat chronic pain, especially very severe pain (Gomółka 1996; Corbett *et al.* 2006).

The periaqueductal grey matter is the main center of the anti-nociceptive system and it has numerous connections with the other CNS structures, of which the most important include: dorsomedial nucleus and ventromedial nucleus of the hypothalamus, nuclei in the medial part of the thalamus, locus ceruleus, raphe nuclei and the reticula formation nuclei. The periaqueductal grey matter, however, does not have any direct connection with the spinal cord. The projections leaving the periaqueductal grey matter and reaching the locus ceruleus, great raphe nucleus and giant cell nucleus give rise to two descending pathways of the inhibitory system: the noradrenergic and serotoninergic pathways. These pathways travel in the dorso-ventral fascicle up to the lamellae I and V of the caudal spinal horn where they stimulate enkephalinergic neurons located in the gelatinous substance. This triggers both presynaptic and postsynaptic inhibition of the second neurons of pain perception (Cui *et al.* 1999; Pertovaara 2006).

Stimulation of the a_2-adrenergic receptors in the noradrenergic system shows synergism in an anti-nociceptive effect with the opioid system at the level of peripheral tissues, spinal cord and supraspinal structures (Snider *et al.* 1998). Studies have demonstrated that the a_2-adrenergic receptors are located within the same spinal, medulla oblongata neurons as the opioid m receptors, and the neural cells of the noradrenergic system found in the pons project reach the opioidergic structures in the grey matter surrounding the chambers (Pan *et al.* 1990). Furthermore, opioids stimulate the noradrenergic descending pathways that originate in the substantia nigra via a presynaptic inhibition of the GABA-nergic ends (Pan *et al.* 2004).

It is important whether a disruption to the adrenergic transmission obtained with DSP-4 administered to neonatal rats impacts the perception of pain stimuli mediated by the 5-HT_3 serotonin receptors at the spinal cord level or higher tiers of the central nervous system.

In previous studies, it was found that following a local injection of formalin to the paw of an animal, the analgesic effect of morphine was reduced in animals with the noradrenergic system lesion caused by DSP-4 (Roczniak *et al.* 2010).

In other studies with FBG, a5-HT_3 serotonin receptor agonist used in the tail immersion test, no differences were observed in the anti-nociceptive effect between a control group and a DSP-4 group. However, with another model of acute pain induced by a thermal stimulus (the hot plate test), FBG, similar to morphine, demonstrated a significantly weaker analgesic effect in animals with the noradrenergic system lesion induced by DSP-4 administration, and this effect was blocked by ondansetron, a 5-HT_3 serotonin receptor antagonist (Roczniak *et al.* 2010).

The studies carried out to date have demonstrated that chemical damage to the central noradrenergic system caused by DSP-4 administered to neonatal rats leads to a significant alteration of the central dopaminergic and serotoninergic transmission as well as to GABA-nergic disturbances in adult rats (Kostrzewa *et al.* 2006; Dąbrowska *et al.* 2007; Bortel *et al.* 2008; Nowak *et al.* 2006; Roczniak *et al.* 2015).

In the investigated model of acute pain caused by a thermal stimulus (the hot plate test and tail immersion test), morphine showed a significantly weaker analgesic effect in animals with a noradrenergic system lesion induced by DSP-4. Similar results were recorded in the test with a mechanical stimulus (the paw withdrawal test).

The tail immersion test was used to evaluate the perception of pain stimulus integration at the spinal cord level, while in the hot plate and paw withdrawal tests the supraspinal analgesic mechanisms are of major importance (Kamali *et al.* 2001; Ressler *et al.* 1999). The tail-flick movement in the tail immersion test was also found in animals with dissected or frozen upper cervical segments of the spinal cord (Jonsson *et al.* 1998).

CONCLUSIONS

Destruction of the central noradrenergic system in an early phase of development resulted in a reduction of the analgesic effect of morphine in the models of acute pain in which the supraspinal mechanism of pain perception is involved.

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